REMARKS/ARGUMENTS

Support for a plant-derived mismatch-directed endonuclease in new independent Claim 91 is found, for example, in the paragraph starting on page 48, line 25, wherein a number of functionally equivalent, and potentially homologous activities to CEL I, found in extracts from a variety of plant species, are cited. Other mismatch-directed endonucleases such as SP nuclease from spinach are cited.

Claims Rejections – 35 USC § 112

Item 2: In the Final Rejection mailed 12/29/05 Claims 62-72, 78-83, 85 and 87-90 were rejected under 35 U.S.C. 112, first paragraph for allegedly containing new matter. The claims have been amended to remove the phrase "defined composition" and now recite simply "enzymes consisting essentially of". In view of the amendment, withdrawal of the new matter rejection is respectfully requested.

Item 3: Claims 62-72, 78-83, 85 and 87-90 were rejected under 35 U.S.C. 112, second paragraph for allegedly being vague and indefinite in the phrase "defined composition containing enzymes wherein the enzymes consist essentially of". The claims have been amended to remove the phrase "defined composition" and now recite simply "enzymes consisting essentially of". In view of the amendment, withdrawal of the rejection is respectfully requested.

Claims Rejections – 35 USC § 102

Item 5: Claims 67, 69-73, 85 and 87-90 were rejected under 35 U.S.C. 102(e) as allegedly anticipated by Vind. Applicants note that Claim 73 has been previously canceled. This rejection is respectfully traversed. Vind attempts to prepare sequence variants by mixing two different double stranded polynucleotides with a whole cell extract containing DNA repair enzymes, heat denaturing the polynucleotides, annealing to form a heteroduplex and allowing the enzymes in the whole cell extract to repair the mismatches thereby producing the parents and sequence variants (see Vind column 2, lines 47-67).

Vind does not anticipate the present claims. The Examiner asserts that the Mut enzyme homologues of Vind possess "strand cleavage activity". However, the "strand cleavage activity" language does not appear in the instant claims. The "mismatch recognizing and mismatch directed endonuclease that cleaves at the mismatched nucleotides" of the instant claims (e.g. CEL I) is not taught by Vind nor is it reasonable to assume it to be inherently present. This enzyme is not part of the normal DNA repair system and therefore not suggested by Vind. The normal bacterial DNA repair system may recognize mismatches but the endonuclease(s) cleave somewhere upstream or downstream from the mismatch. Therefore, Vind's DNA repair system's endonuclease is neither "mismatch directed", nor does it "cleave at the mismatched nucleotides".

To further clarify the distinction between the instant invention and systems relying on DNA repair systems Applicants have submitted the Rule 132 Declaration of Padgett. It is clear from the Declaration that treatment of a heteroduplex with a DNA repair system is the equivalent of the negative control (background) of the instant invention. The highly reassorted output molecules of the instant invention are very different from the output molecules a person of ordinary skill in the art would expect to obtain after treatment of a heteroduplex with a DNA repair system. Vind fails to anticipate the dependent Claims 69-73, 85 and 87-90 for at least the reasons detailed for the independent Claim 67. Reconsideration and withdrawal of the rejection is respectfully requested.

New Claims 91-105 are not anticipated by Vind at least for the reasons detailed above. In addition, Vind does not teach or suggest "a **plant-derived** mismatch recognizing and mismatch directed endonuclease that cleaves at the mismatched nucleotides" as required in the new claims.

Claims Rejections – 35 USC § 103

Items 6-8: Claim 68 was rejected under 35 U.S.C 103(a) as allegedly unpatentable over Vind. This rejection is respectfully traversed. For at least the reasons detailed above, Vind does not teach the instant invention. In addition, Vind does not teach adding the ingredients in the order claimed in Claim 68. The whole point of the method of Vind is to use a DNA repair system, specifically a thermostable DNA repair

system, such that all of the ingredients are present when he forms the heteroduplex by heat denaturation of the parental polynucleotides. Indeed, Vind is not motivated to make **any** order of addition since he relies upon an intact DNA repair system to perform his reaction. For all of these reasons, reconsideration and withdrawal of the rejection is respectfully requested.

Item 9: Claims 75-77 and 80 were rejected under 35 U.S.C. 103(a) as unpatentable over Vind in view of Arnold. Applicants note that Claims 75-77 were previously canceled. This rejection is respectfully traversed. Arnold et al. is cited to show another DNA repair system used for mismatch correction using *E. coli* extracts which presumably contain *E. coli* Pol 1. The Examiner argues that to substitute equivalents between the two systems would be obvious. The Rule 132 Declaration by Padgett shows the distinction between the instant invention and systems relying upon a DNA repair system such as that described by Vind and Arnold.

Adding Arnold et al. does nothing to overcome the deficiencies of Vind referred to in responding to the rejections over Vind alone. This is especially so with respect to the "mismatch recognizing and mismatch directed endonuclease that cleaves at the mismatched nucleotides" (e.g. CEL I) which is not hinted at in Arnold et al. Claim 80 recites that the enzyme with both polymerase activity and 3' to 5' exonuclease activity is *E. coli* Pol I. This enzyme is not present in anything other than a whole cell or cell extract and therefore is not available for adding to the Vind DNA repair system. While Arnold et al. may contain the same enzyme in the cells used in their DNA repair system, one would not be motivated to simply pull it out and add it to the Vind DNA repair system because *E. coli* Pol I would not operate in the Vind method. Vind specifically recites using thermostable enzymes. The ordinary skilled artisan knows that *E. coli* Pol I is NOT thermostable. Vind subjects his enzymes to temperatures sufficiently high to melt and anneal the polynucleotide strands into a heteroduplex. This temperature would inactivate *E. coli* Pol I. Note the examples expose the enzymes (cell extract) to PCR conditions of 95° C and 72° C, clearly too hot for *E. coli* Pol I. Therefore, one lacks motivation to combine the references in the manner suggested by the examiner because it would be expected to be inoperable.

The Examiner has argued that Vind has a preferred embodiment of using high temperature enzymes but this does not prevent the use of alternative embodiments. However, this mischaracterizes Vind. All embodiments of Vind require high temperature enzymes because all embodiments in Vind subject the enzymes to temperatures sufficiently high to melt and anneal double stranded polynucleotides. The Examiner has pointed to Vind column 5, lines 50-56 as listing enzymes that are not thermostable. However, reading the entire sentence it is clear that it is thermostable **homologues** of the enzymes that are being discussed. This is further clarified in Vind column 8, lines 9-26 wherein the thermostable homologues are further discussed. Vind provide **no** teaching or suggestion as to how his method could be practiced without thermostable enzymes. Vind teaches no alternative to thermal denaturation of the double-stranded polynucleotides for preparation of the heteroduplex that would allow non-thermostable enzymes to survive the treatment. In his Examiner's Answer mailed December 5, 2006 the Examiner argues:

> "While the Vind method prefers to perform multiple repeating steps of denaturation and recombination, Vind does not require repetition, as expressly indicated by the "optional" nature of the repetition at column 2, lines 65-68. Consequently, when Vind performs the method in a single step, the enzyme need not be thermostable since it will not need to survive multiple rounds of amplification."

This is a mischaracterization of the method of Vind. There is no substrate for the method of Vind until the formation of the heteroduplex. The heteroduplex is formed by thermal denaturation followed by annealing (see Vind column 2, lines52-63). Hence, **before even one round** of the reaction has been performed, the enzymes from the cell extract have been subjected to thermal denaturation. Hence it would **not** be obvious to add the *E. coli* Pol I enzyme of Arnold et al. to the single round embodiment of Vind since the Pol I would likely be inactivated in the initial formation of the heteroduplex. For all of these reasons, reconsideration and withdrawal of the rejection is respectfully requested.

Item 10: Claims 78 and 79 were rejected under U.S.C. 103(a) as unpatentable over Vind in view of Birkenkamp et al. This rejection is respectfully traversed. As discussed above, Vind does not anticipate the instant invention. In addition, substitution of the T4

enzymes of Birkenkamp et al. into the method of Vind would not yield the instant invention. Indeed, a person of ordinary skill in the art would expect the addition of T4 enzymes into the method of Vind to have no effect, as the enzymes would likely be inactivated in the preliminary step of thermal denaturation to form the heteroduplex in the single round embodiment. Reconsideration and withdrawal of the rejection is respectfully requested.

Item 11: Claims 66-74, 81-82, 85 and 87-90 are rejected under 35 U.S.C. 103(a) as being unpatentable over Vind in view of Oleykowski et al. This rejection is respectfully traversed. Vind fails to teach the elements of Claims 67-73 and 85 and 87-90 as discussed above. With regard to Claims 66, 81 and 82, the addition of Oleykowski et al. does not compensate for the deficiencies of Vind. In addition, the substitution of CEL I into the method of Vind would be expected by one of ordinary skill in the art to fail for similar reasons as the enzymes of Arnold et al. and Birkenkamp et al. as discussed above. There is no substrate for the method of Vind until the formation of the heteroduplex. The heteroduplex is formed by thermal denaturation. Hence, **before even one round** of the reaction has been performed, the enzymes from the cell extract have been subjected to thermal denaturation. Hence it would **not** be obvious to add CEL I enzyme of Oleykowski et al. to the single round embodiment of Vind since the CEL I would likely be inactivated in the initial formation of the heteroduplex. Since the references, alone or in combination, fail to teach or suggest all the elements of the claimed invention, reconsideration and withdrawal of the rejection is respectfully requested.

In view of the amendments and comments above, the rejections have been overcome. Reconsideration, withdrawal of the rejections and early indication of allowance are respectfully requested. If any issues remain, the examiner is encouraged to call the undersigned for prompt resolution.

If needed, Applicants petition for an extension of time sufficient for consideration of this response.

Please charge the fees associated with filing the Request for Continued Examination to Deposit Account No.500933.

The commissioner hereby is authorized to charge payment of any fees under 37 CFR § 1.17, which may become due in connection with the instant application or credit any overpayment to Deposit Account No.500933.

Respectfully submitted,

Wayne Fitzmaurice

Date: October 26, 2007

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Enclosures: RCE Transmittal (Two copies of 1 page)
Declaration by Hal S. Padgett, Ph.D. (14 pages)
Copies of references in Declaration (10 documents, 68 pages)
Figures supporting Declaration (5 sheets)
Self-addressed postcard

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